

CLAIMS

We claim:

1. A method for making a transcription product corresponding to a target sequence in a target nucleic acid, the method comprising:
 - (a) obtaining a target nucleic acid;
 - (b) obtaining a sense promoter primer, the sense promoter primer comprising a 5'-end portion comprising a sense transcription promoter and a 3'-end portion that is complementary to the target;
 - (c) annealing the sense promoter primer with the target nucleic acid so as to form a target nucleic acid-sense promoter primer complex;
 - (d) contacting the target nucleic acid-sense promoter primer complex with a DNA polymerase under polymerization reaction conditions so as to obtain first-strand cDNA that is complementary to the target sequence;
 - (e) obtaining first-strand cDNA;
 - (f) ligating the first-strand cDNA under ligation conditions so as to obtain circular sense promoter-containing first-strand cDNA;
 - (g) obtaining an anti-sense promoter oligo;
 - (h) annealing the anti-sense promoter oligo to the circular sense promoter-containing first-strand cDNA so as to obtain a circular transcription substrate;
 - (i) obtaining the circular transcription substrate; and
 - (j) contacting the circular transcription substrate with an RNA polymerase under transcription conditions, wherein a transcription product is obtained.
2. The method of claim 1 wherein the anti-sense promoter oligo comprises an oligo that is immobilized on a solid support.

3. A method for detecting an analyte in or from a sample, the method comprising:
- a) obtaining an analyte-binding substance-oligonucleotide ("ABS-oligo"), wherein the ABS-oligo comprises an ABS that is joined to a oligonucleotide comprising a sequence for an anti-sense promoter portion of a double-stranded promoter for an RNA polymerase that recognizes the promoter;
 - b) obtaining a Signal Probe, wherein the Signal Probe comprises a sense promoter that is joined to the 3'-end of a template, wherein the sense promoter is sufficiently complementary to the anti-sense promoter of the ABS-oligo to form a complex that can be used for transcription of the template using an RNA polymerase that binds to the complex;
 - c) contacting an ABS-oligo with a surface to which an analyte is bound if present in a sample under analyte-binding conditions that permit the ABS-oligo to bind the analyte if present on said surface;
 - d) washing the surface under conditions that permit removal of unbound ABS-oligo;
 - e) contacting the surface with a Signal Probe under complexing conditions that permit complexing of the Signal Probe with the ABS-oligo if present on the surface;
 - f) optionally, washing the surface under conditions that permit removal of unbound Signal Probe;
 - g) contacting the surface with an RNA polymerase under conditions that permit transcription of a product encoded by the template using the complex between the ABS-oligo and the Signal Probe; and
 - h) detecting a transcription product encoded by the template, if present.

4. A method for amplifying the amount of a template-complementary transcription product, the method comprising:
- a) obtaining a transcription product;
 - b) obtaining a sense promoter primer comprising a 3'-end portion that is complementary to the 3'-end of the transcription product and optionally, a phosphate group or a topoisomerase moiety on its 5-end;
 - c) annealing the sense promoter primer to the transcription product;
 - d) primer-extending the promoter primer annealed to the transcription product with an RNA-dependent DNA polymerase under DNA synthesis conditions so as to obtain first-strand cDNA;
 - e) optionally, removing the RNA that is annealed to the first-strand cDNA;
 - f) ligating the first-strand cDNA, wherein the 5'-end is covalently joined to the 3'-end of the first-strand cDNA so as to obtain circular sense promoter-containing first-strand cDNA;
 - g) annealing an anti-sense promoter oligo to the circular sense promoter-containing first-strand cDNA so as to obtain a circular substrate for transcription;
 - h) contacting the circular substrate for transcription with an RNA polymerase under transcription conditions so as to obtain additional transcription product; and
 - i) obtaining the additional transcription product.
5. The method of claim 4 wherein the sense promoter primer comprises a single-stranded sense promoter chosen from the group consisting of a pseudopromoter or a synthetic promoter that is used by an RNA polymerase to make additional transcription product and wherein an anti-sense promoter oligo is not used to obtain additional transcription product.
6. The method of claim 4 wherein the sense promoter primer comprises an N4 promoter and the RNA polymerase used for transcription is chosen from among N4 vRNAP, mini-vRNAP, and a mutant mini-vRNAP, and wherein an anti-sense promoter oligo is not used to obtain additional transcription product.